

## **Supplementary Information**

### **The receptor PTPRU is a redox sensitive pseudophosphatase**

Iain M. Hay, Gareth W. Fearnley, Pablo Rios, Maja Köhn, Hayley J. Sharpe and Janet E. Deane

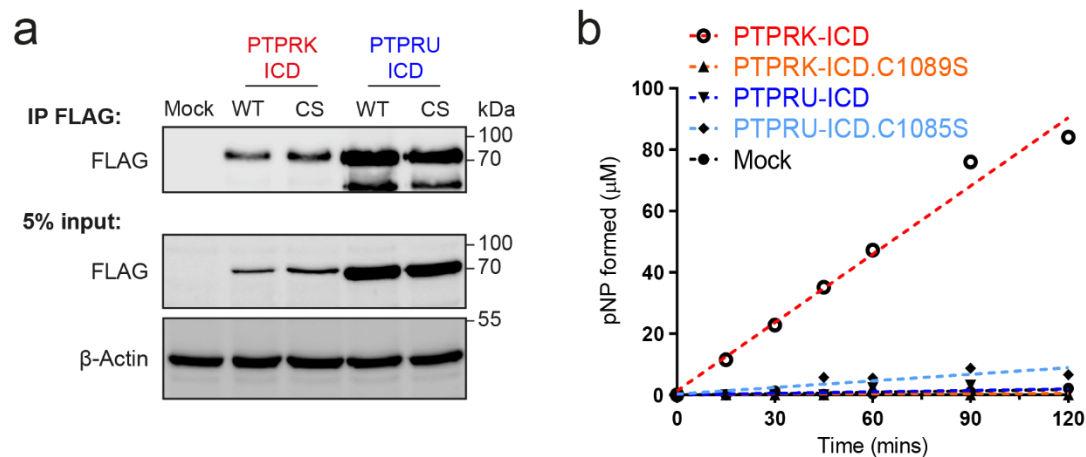
Supplement contains 10 supplementary figures, 3 supplementary tables and supplementary references.

Family	PTP	pTyr-loop	WPD-loop	PTP-loop
R1	PTPRC	677-686 NQNKNRYVDI	816-824 FTSWPDHGV	850-861 VVHCSAGVGRTG
	PTPRM	923-932 NRMKNRYGNI	1058-1066 FTGWPDHGV	1092-1103 VVHCSAGAGRTG
R2B	PTPRK	911-920 NRAKNRYGNI	1052-1060 FTGWPDHGV	1086-1097 VVHCSAGAGRTG
	PTPRT	912-921 NRNKNRYGNI	1047-1055 FTSWPDHGV	1081-1092 VVHCSAGAGRTG
	PTPRU	913-922 KVKGSRQEPM	1048-1056 FTAWPEHGV	1082-1093 VVHCSAGTGRG
R2A	PTPRF	1375-1384 NKPKNRYANV	1511-1519 FMAWPDHGV	1545-1556 VVHCSAGVGRTG
	PTPRS	1416-1425 NKPKNRYANV	1552-1560 FTAWPDHGV	1586-1597 VVHCSAGVGRTG
	PTPRD	1380-1389 NKPKNRYANV	1516-1524 FTAWPDHGV	1550-1561 VVHCSAGVGRTG
R4	PTPRA	265-274 NKEKNRYVNI	405-413 FTSWPDFGV	439-450 VVHCSAGVGRTG
	PTPRE	159-168 NREKNRYPNI	298-306 FTSWPDFGV	332-343 VVHCSAGVGRTG
R5	PTPRG	874-883 NKHKNRYINI	1023-1031 YTWPDHGV	1057-1068 LVHCSAGVGRTG
	PTPRZ	1750-1759 NKHKNRYINI	1896-1904 YTWPDHGV	1930-1941 VVHCSAGVGRTG
	PTPRB	1727-1736 NRGKNRYNNI	1865-1873 YTVWPDHGV	1901-1912 VVHCSAGVGRTG
R3	PTPRJ	1065-1074 NRGKNRYNNV	1200-1208 FTSWPDHGV	1236-1247 LVHCSAGVGRTG
	PTPRH	844-853 NNAKNRYRNV	981-989 YQAWPDHGV	1017-1028 LVHCSAGVGRTG
	PTPRO	962-971 NRCKNRYTNI	1097-1105 YTAWPDHGV	1133-1144 LVHCSAGVGRTG
R7	PTPRQ	2060-2069 NRAKNRFPMI	2196-2204 FTAWPEHGV	2230-2241 LVHCSAGVGRTG
	PTPRR	415-424 HGTKNRYKTI	549-557 YTSWPDHKT	585-596 VVHCSAGIGRTG
	PTN5	322-331 LVRKNRYKTI	456-464 FTSWPDQKT	493-504 LVHCSAGIGRTG
R8	PTPRN	734-743 NIKKNRHPDF	872-880 FLSWPAEGT	906-917 LVHCSAGAGRTG
	PTPRN2	770-779 NVPKNRSLAV	908-916 FLSWYDRGV	942-953 LVHCSAGAGRSG
NT1	PTN1	40-49 NKNRNRYRDV	176-184 YTTWPDHGV	212-223 VVHCSAGIGRSG
	PTN2	42-51 NRNRNRYRDV	177-185 YTTWPDHGV	213-224 LVHCSAGIGRSG
NT2	PTN6	270-279 NKGKNRYKNI	414-422 YLSWPDHGV	450-461 LVHCSAGIGRTG
	PTN11	273-282 NKNKNRYKNI	424-432 FRTWPDHGV	460-471 VVHCSAGIGRTG
NT3	PTN9	327-336 NLEKNRYGDV	465-473 FLSWPDYGV	512-523 VVHCSAGIGRTG
	PTN18	56-65 NVRKNRYKDV	192-200 YMSWPDHGV	226-237 CVHCSAGCGRTG
NT4	PTN12	58-67 NVKKNRYKDI	194-202 YVNWPDHGV	228-239 CVHCSAGCGRTG
	PTN22	54-63 NIKKNRYKDI	190-198 YKNWPDHGV	224-235 CVHCSAGCGRTG
NT5	PTN3	670-679 NLDKNRYKDV	806-814 YVAWPDHGV	839-850 LVHCSAGIGRTG
	PTN4	679-688 NISKNRYRDI	815-823 YIAWPDHGV	849-860 VVHCSAGIGRTG
NT6	PTN21	921-930 NAERNRFQDV	1062-1070 YTDWPEHGC	1105-1116 LVHCSAGVGRTG
	PTN14	933-942 NAERSRIREV	1074-1082 YTDWPDHGC	1118-1129 VVHCSAGVGRTG
NT7	PTN13	2237-2246 NRRKNRYKNI	2373-2381 FTAWPDHDT	2405-2416 LVHCSAGIGRSG
NT8	PTN23	1217-1226 YSLKNRHQDV	1352-1360 FPTWPELGL	1389-1400 LVHCSAGVGRTG
NT9	PTN20	183-192 NREKNRYRDI	318-326 FTKWPDHGT	350-361 VVHCSAGIGRTG
	PTN7	119-128 HASKDRYKTI	252-260 FSAWPDHQT	288-299 VVHCSAGIGRTG

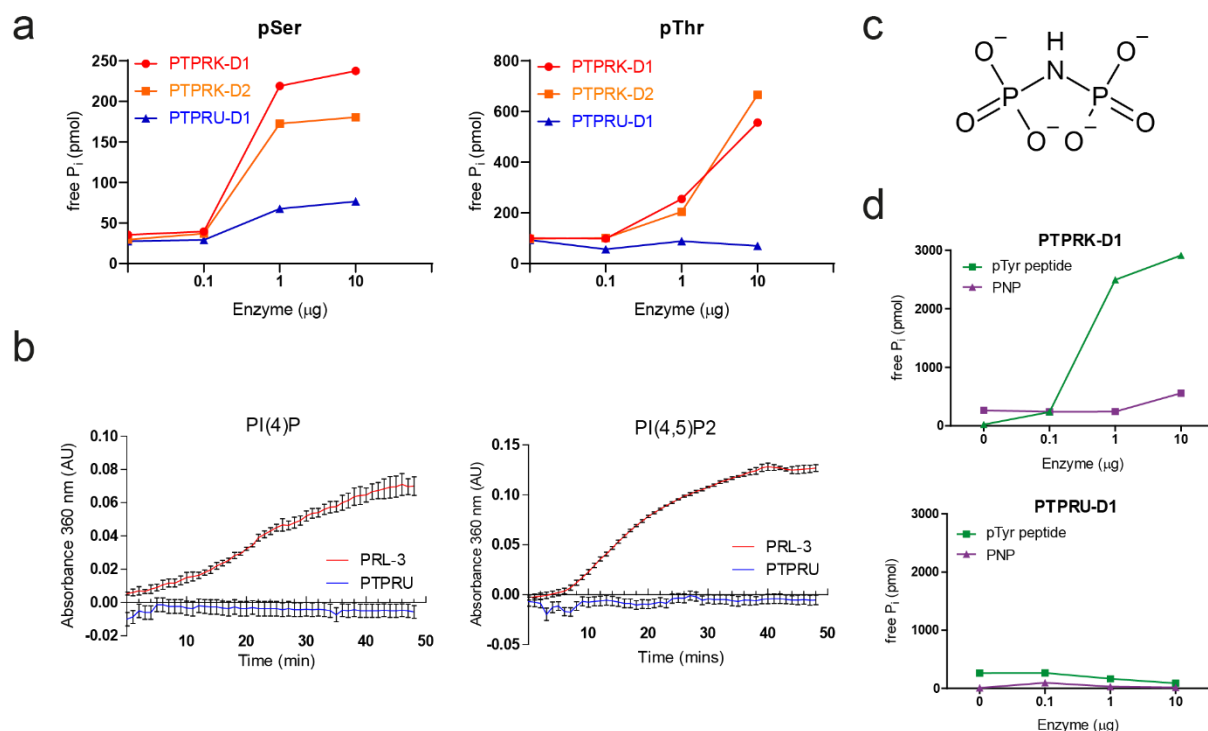
**Supplementary Fig. 1.** Multiple sequence alignment of the pTyr recognition loop, WPD loop and PTP loop of the 37 classical PTPs, coloured by percentage identity (blue). Key variable residues in PTPRU are highlighted in red.

				pTyr recognition loop			
PTPRU_HUMAN/871-1153	871	HPAVRVADLLQHINQMKTAEYGF	KQEYESF	EGWDATKKKDKVKGS	RQEPMPAY	DRHRVKLHPMLGDP	NADYI
PTPRU_MOUSE/871-1153	871	HPAVRVADLLQHINQMKTAEYGF	KQEYESF	EGWDATKKKDKVKGS	RQEPVSA	DRHRVKLHPMLADPD	ADYI
PTPRU_DOG/871-1153	871	HPAVRVADLLQHINQMKTAEYGF	KQEYESF	EGWDATKKKDKVKGS	RQEP	DRHRVKLPPMMGGP	ADYI
PTPRU_CHICK/860-1141	860	HPAVRVADLLQHINQMKTAEYGF	KQEYESF	EGWDASKKKDKTKG	-RQDHVSTY	DRHRVKLHPLLGD	PNSDYI
PTPRU_FISH/870-1151	870	HPAVRVADLLQHINQMKTAEYGF	KQEYESF	DGWDINKKKDKTKG	-RHD	TLMGYDRHRVKLHPLLGD	PNSDYI
*							
PTPRU_HUMAN/871-1153	945	NANYIDGYHRSNHFIATQGPKP	EMVYDFWRMVWQEHCSS	IVMITKLVEVGRVKCSRYWP	EDSDTYGDIK	ITL	VK
PTPRU_MOUSE/871-1153	945	SANYIDGYHRSNHFIATQGPKP	EMVYDFWRMVWQEHCSS	IVMITKLVEVGRVKCSRYWP	EDSDMYGDIK	ITL	VK
PTPRU_DOG/871-1153	945	NANYIDGYHRSNHFIATQGPKP	EMVYDFWRMVWQEHCSS	IVMITKLVEVGRVKCSRYWP	EDSEMYGDIK	ITL	VK
PTPRU_CHICK/860-1141	933	NANYIDGYHRSNHFIATQGPKQ	EMVYDFWRMVWQEHCSS	IVMITKLVEVGRVKCSKYWP	DDSEMYGDIK	ITL	VK
PTPRU_FISH/870-1151	943	NANYIDGYHRSNHFIATQGPKQ	ETVYDFWRMVWQENCF	SVMITKLVEVGRVKCKYWP	DESEMYGDIK	ITL	VK
				WPD loop		PTP loop	
PTPRU_HUMAN/871-1153	1019	TETLAEYVVRTFALERRGYSARHEV	RQFHFT	AWPEHGV	PYHATGLLAFIRRVKASTPPDAGP	IVIHCSAGT	GR
PTPRU_MOUSE/871-1153	1019	TETLAEYVVRTFALERRGYSARHEV	RQFHFT	AWPEHGV	PYHATGLLAFIRRVKASTPPDAGP	IVIHCSAGT	GR
PTPRU_DOG/871-1153	1019	TETLAEYVVRTFALERRGYSARHEV	RQFHFT	AWPEHGV	PYHATGLLAFIRRVKASTPPDAGP	IVIHCSAGT	GR
PTPRU_CHICK/860-1141	1007	SEMLAEYAVRTFALERRGYSARHEV	KQFHFT	SWPEHGV	PYHATGLLAFIRRVKASTPPDAGP	IVIHCSAGT	GR
PTPRU_FISH/870-1151	1017	TETLAEYVVRTFALERRGYSARKHEV	CQFHFT	SWPEHGV	PYHATGLLAFIRRVKSTPLDAGP	VVHCSV	GAGRT
Q loop							
PTPRU_HUMAN/871-1153	1093	GCYIVLDVMLDMAECEGVVDIYNCVK	TLCSR	RVNMIQT	EEQYIF	IHDAILEACL	CGETTIP
PTPRU_MOUSE/871-1153	1093	GCYIVLDVMLDMAECEGVVDIYNCVK	TLCSR	RVNMIQT	EEQYIF	IHDAILEACL	CGETTIP
PTPRU_DOG/871-1153	1093	GCYIVLDVMLDMAECEGVVDIYNCVK	TLCSR	RVNMIQT	EEQYIF	IHDAILEACL	CGETTIP
PTPRU_CHICK/860-1141	1081	GCYIVLDVMLDMAECEGVVDIYNCVK	TLCSR	RVNMIQT	EEQYIF	IHDAILEACL	CGETTIP
PTPRU_FISH/870-1151	1091	GCYIVLDVMLDMAECEGVVDIYNCVK	TLCSR	RVNMIQT	EEQYIF	IHDAILEACL	CGETTIP

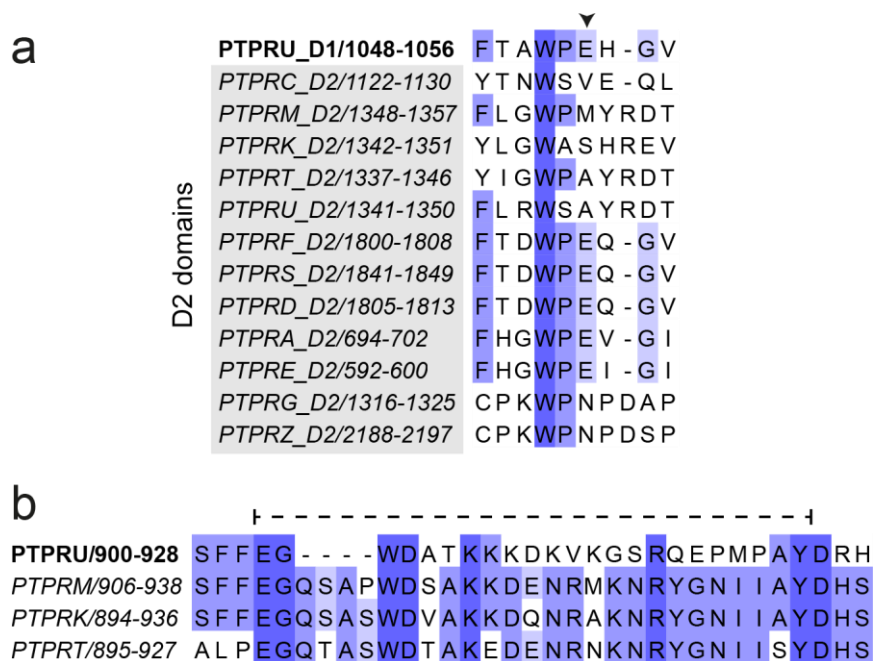
**Supplementary Fig. 2.** Multiple sequence alignment of PTPRU-D1 sequences from human (*Homo sapiens*), mouse (*Mus musculus*), dog (*Canis familiaris*), chicken (*Gallus gallus*) and zebrafish (*Danio rerio*). Sequences are coloured by percentage identity (blue). Key PTP motifs are labelled and boxed in red. The “backdoor” cysteine (C998) which upon oxidation forms an intramolecular disulphide with the catalytic C1085 of the PTP loop is highlighted (\*).



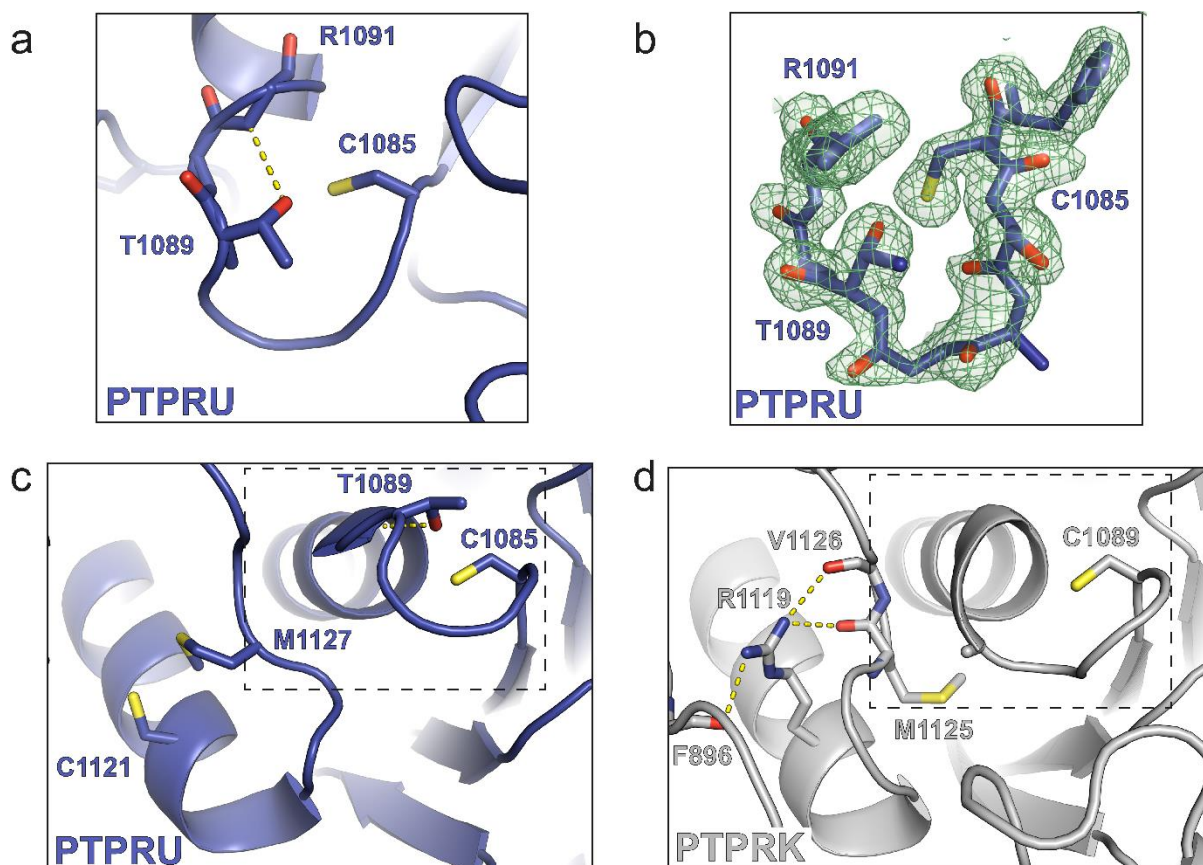
**Supplementary Fig. 3. Replicate of immunoprecipitation (IP) and pNPP assays of FLAG-tagged PTP intracellular domains (ICD).** **a** Immunoblot analysis of FLAG IPs from HEK-293T cells transiently transfected with PTPRK and PTPRU WT and CS inactivating mutant ICDs. **b** Time course of pNPP dephosphorylation by FLAG IPs from (a).



**Supplementary Fig. 4. The PTPRU-D1 domain shows no activity against diverse substrates.** **a** Activity of PTPRU-D1, PTPRK-D1 and PTPRK-D2 domains incubated with 100 μM of either pSer or pThr amino acids, measured using BIOMOL Green reagent. Note: the activity levels detected here are very low compared to that of a validated enzyme-substrate reaction (compare to Y axis of panel **d**). The equivalent activity of the PTPRK D1 and D2 domains suggests this level of activity is non-specific. **b** Phosphatidylinositol (PI) phosphatase activity assay. Phosphatidylinositol 4-phosphate [PI(4)P] and phosphatidyl 4,5-bisphosphate [PI(4,5)P2] substrates were incubated with either 3 μM PTPRU-D1 or 6 μM PRL-3 (positive control for PI phosphatase activity)<sup>1</sup> and product formation monitored by measurement of absorbance at 360 nm. **c** Chemical structure of the phosphoramidate-linked substrate imidodiphosphate (PNP). **d** Activity of PTPRU and PTPRK D1 domains incubated with 100 μM of either pTyr peptide or PNP, measured using BIOMOL Green reagent.

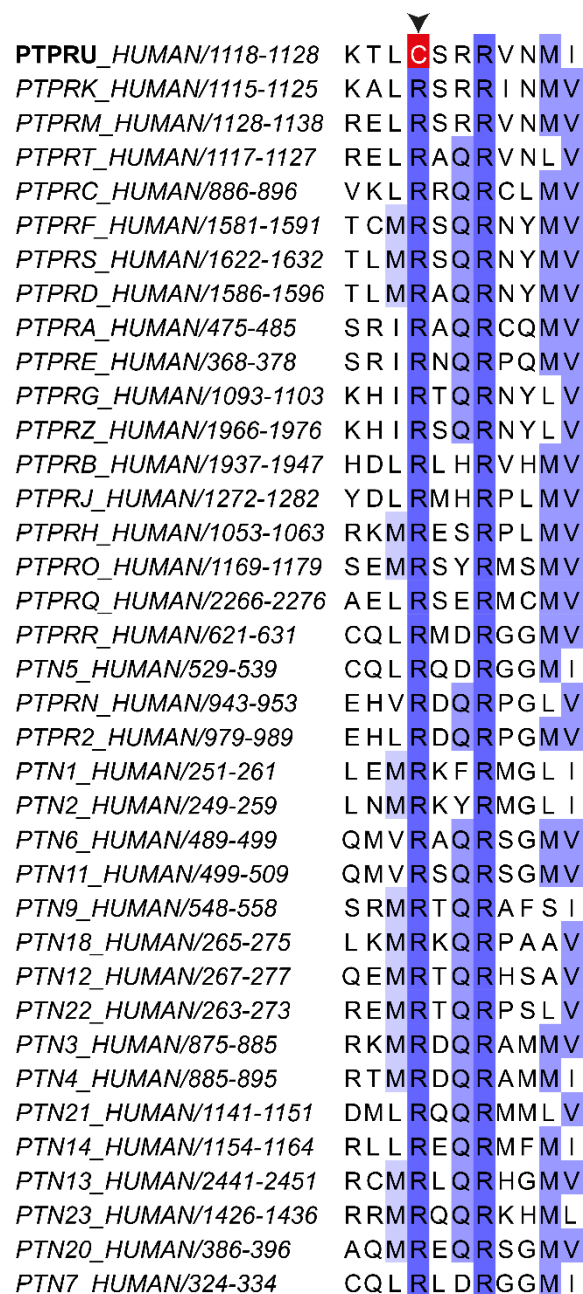


**Supplementary Fig. 5. Sequence alignments of PTPRU catalytic motifs with relevant PTPs. a** Multiple sequence alignment of the PTPRU-D1 WPD loop with those of the receptor PTP D2 domains, coloured by percentage identity (blue). The non-canonical glutamate of the PTPRU-D1 WPD-loop is marked by an arrowhead. **b** Multiple sequence alignment of the R2B family pTyr recognition loops coloured by percentage identity (blue). Residues 904-925 of PTPRU, which are disordered in the structure, are highlighted by a dashed line.



**Supplementary Fig. 6. Conformational changes of the PTP and adjacent loops.** **a** T1089 within the PTP loop is stabilised in a novel conformation via hydrogen bonding to the backbone of R1091, capping the end of the  $\alpha$ -helix. Hydrogen bonds are illustrated by a dotted yellow line. For clarity only specific mainchain and sidechain atoms are shown to help illustrate relevant bonds and interactions. **b** Electron density ( $2F_o - F_c$  contoured at  $0.8 \text{ e}^-/\text{\AA}^3$ , green) for the novel PTPRU-D1 PTP loop (H1084-R1091, blue sticks) conformation shown in (a). **c** In PTPRU, the loop C1121-M1127 adjacent to the PTP loop adopts a conformation that differs from classical PTPs due to re-orientation of M1127. This loop is not well ordered and was challenging to build in a single conformation suggesting it may adopt multiple conformations. The dotted inset box identifies the equivalent region as illustrated in Fig. 2d in the main text. **d** In PTPRK, the equivalent loop R1119-M1125 is stabilised via hydrogen bonding between the R1119 sidechain and backbone carboxyl groups of M1125 and V1126. As for panel (c), the dotted inset box identifies the equivalent region as illustrated in Fig. 2d.

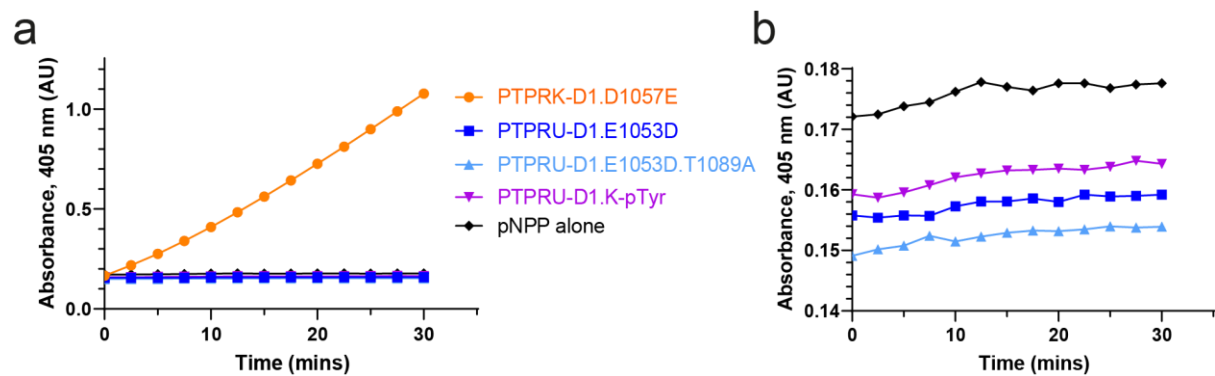




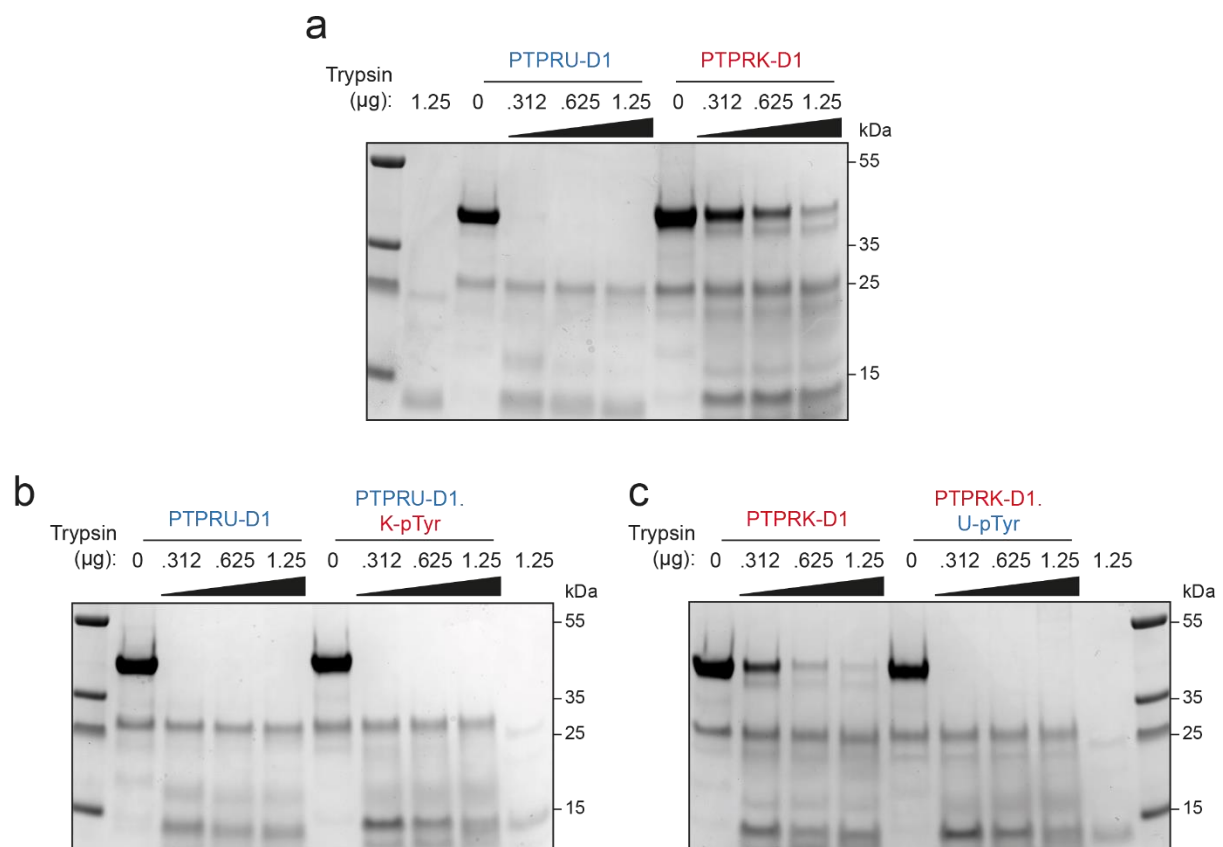
<b>PTPRU_HUMAN/1118-1128</b>	K T L <b>C</b> S R R V N M I
<i>PTPRK_HUMAN/1115-1125</i>	K A L R S R R I N M V
<i>PTPRM_HUMAN/1128-1138</i>	R E L R S R R V N M V
<i>PTPRT_HUMAN/1117-1127</i>	R E L R A Q R V N L V
<i>PTPRC_HUMAN/886-896</i>	V K L R R Q R C L M V
<i>PTPRF_HUMAN/1581-1591</i>	T C M R S Q R N Y M V
<i>PTPRS_HUMAN/1622-1632</i>	T L M R S Q R N Y M V
<i>PTPRD_HUMAN/1586-1596</i>	T L M R A Q R N Y M V
<i>PTPRA_HUMAN/475-485</i>	S R I R A Q R C Q M V
<i>PTPRE_HUMAN/368-378</i>	S R I R N Q R P Q M V
<i>PTPRG_HUMAN/1093-1103</i>	K H I R T Q R N Y L V
<i>PTPRZ_HUMAN/1966-1976</i>	K H I R S Q R N Y L V
<i>PTPRB_HUMAN/1937-1947</i>	H D L R L H R V H M V
<i>PTPRJ_HUMAN/1272-1282</i>	Y D L R M H R P L M V
<i>PTPRH_HUMAN/1053-1063</i>	R K M R E S R P L M V
<i>PTPRO_HUMAN/1169-1179</i>	S E M R S Y R M S M V
<i>PTPRQ_HUMAN/2266-2276</i>	A E L R S E R M C M V
<i>PTPRR_HUMAN/621-631</i>	C Q L R M D R G G M V
<i>PTN5_HUMAN/529-539</i>	C Q L R Q D R G G M I
<i>PTPRN_HUMAN/943-953</i>	E H V R D Q R P G L V
<i>PTPR2_HUMAN/979-989</i>	E H L R D Q R P G M V
<i>PTN1_HUMAN/251-261</i>	L E M R K F R M G L I
<i>PTN2_HUMAN/249-259</i>	L N M R K Y R M G L I
<i>PTN6_HUMAN/489-499</i>	Q M V R A Q R S G M V
<i>PTN11_HUMAN/499-509</i>	Q M V R S Q R S G M V
<i>PTN9_HUMAN/548-558</i>	S R M R T Q R A F S I
<i>PTN18_HUMAN/265-275</i>	L K M R K Q R P A A V
<i>PTN12_HUMAN/267-277</i>	Q E M R T Q R H S A V
<i>PTN22_HUMAN/263-273</i>	R E M R T Q R P S L V
<i>PTN3_HUMAN/875-885</i>	R K M R D Q R A M M V
<i>PTN4_HUMAN/885-895</i>	R T M R D Q R A M M I
<i>PTN21_HUMAN/1141-1151</i>	D M L R Q Q R M M L V
<i>PTN14_HUMAN/1154-1164</i>	R L L R E Q R M F M I
<i>PTN13_HUMAN/2441-2451</i>	R C M R L Q R H G M V
<i>PTN23_HUMAN/1426-1436</i>	R R M R Q Q R K H M L
<i>PTN20_HUMAN/386-396</i>	A Q M R E Q R S G M V
<i>PTN7_HUMAN/324-334</i>	C Q L R L D R G G M I

**Supplementary Fig. 7.** Multiple sequence alignment of PTPRU-D1 loop K1118-I1128 across the 37 classical PTPs, coloured by percentage identity (blue). C1121 (black arrowhead) in this loop is uniquely a cysteine in PTPRU.

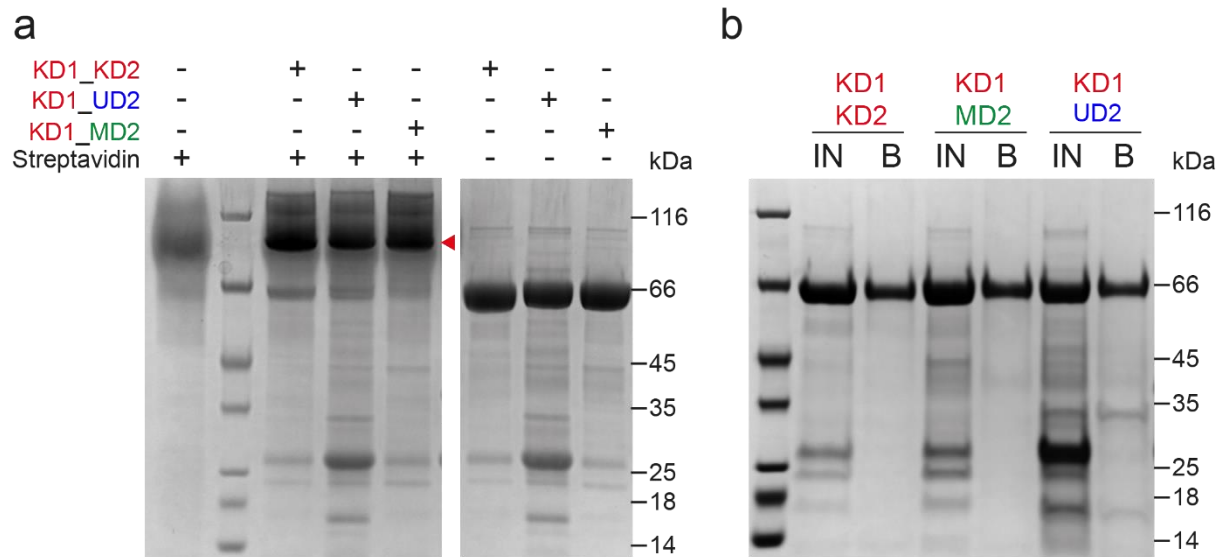




**Supplementary Fig. 8. pNPP activity assays of PTPRU-D1 mutants.** **a** Time course of pNPP dephosphorylation, monitored by absorbance at 405 nm, using 10  $\mu$ M of PTPRK-D1.D1057E, PTPRU-D1.E1053D, PTPRU-D1.E1053D.T1089A and PTPRU-D1.K-pTyr recombinant proteins. **b** pNPP dephosphorylation data of inactive PTPRU-D1 mutants from (a). Background absorbance for all proteins does not exceed that observed for pNPP substrate incubated with assay buffer alone.



**Supplementary Fig. 9. Trypsin limited proteolysis of the D1 domain pTyr recognition loop chimeras.** Limited proteolysis of **a** PTPRU-D1 and PTPRK-D1, **b** PTPRU-D1 and PTPRU-D1.K-pTyr, **c** PTPRK-D1 and PTPRK-D1.U-pTyr with trypsin followed by SDS-PAGE and Coomassie staining.



**Supplementary Fig. 10.** *In vivo* biotinylation of chimeric tandem PTP domains. **a** *In vivo* biotinylated chimeric tandem PTP domains incubated with or without streptavidin, resolved by SDS-PAGE and visualized by Coomassie staining. Mobility shift upon streptavidin binding to biotinylated protein is indicated by arrowhead. **b** *In vivo* biotinylated chimeric tandem PTP domains bound to streptavidin magnetic beads. Input protein (IN) and protein eluted from washed beads (B) was resolved by SDS-PAGE and visualized by Coomassie staining.

**Supplementary Table 1.** Percentage sequence identity matrix of R2B family D1 domains vs R2B family D1 (green) and D2 (yellow) domains. Generated by multiple sequence alignment using Clustal Omega<sup>2</sup>.

	PTPRU D1	PTPRK D1	PTPRM D1	PTPRT D1	PTPRU D2	PTPRK D2	PTPRM D2	PTPRT D2
PTPRU D1	100	72.44	69.61	64.66	27.34	29.2	28.15	26.64
PTPRK D1	72.44	100	79.44	76.31	28.37	31.29	31.02	28.78
PTPRM D1	69.61	79.44	100	80.14	27.66	30.58	30.29	27.34
PTPRT D1	64.66	76.31	80.14	100	29.79	30.58	30.29	29.14

**Supplementary Table 2.** PTP domain structures used in structural alignments (Fig 2b and 2d).

PTP	PDB ID	RMSD (Å)*
PTPN5	2BIJ	1.0 over 1010 atoms
PTPN6	4HJP	1.0 over 1082 atoms
PTPN11	3B7O	1.0 over 1121 atoms
PTPN9	2PA5	0.9 over 1208 atoms
PTPN18	2OC3	1.2 over 1029 atoms
PTPN22	2P6X	1.2 over 1085 atoms
PTPN3	2B49	1.0 over 1139 atoms
PTPN4	2I75	1.1 over 1121 atoms
PTPN14	2BZL	1.1 over 1102 atoms
PTPN13	1WCH	1.0 over 1038 atoms
PTPRC	1YGU	1.1 over 1255 atoms
PTPRF	1LAR	0.8 over 1233 atoms
PTPRS	2FH7	0.9 over 1446 atoms
PTPRM	1RPM	0.8 over 1505 atoms
PTPRK	2C7S	0.8 over 1582 atoms
PTPRT	2OOQ	0.9 over 1500 atoms
PTPRB	2AHS	1.0 over 1337 atoms
PTPRJ	2NZ6	1.0 over 1216 atoms
PTPRO	2GJT	1.1 over 1332 atoms
PTPRA	1YFO	0.9 over 1334 atoms
PTPRE	2JJD	0.9 over 1204 atoms
PTPRG	2H4V	1.0 over 1416 atoms
PTPRR	2A8B	1.2 over 1170 atoms
PTPN7	2A3K	1.0 over 1095 atoms
PTPRN	2I1Y	1.1 over 1142 atoms
PTPRN2	2QEP	0.9 over 1062 atoms
PTPN1	2NT7	1.2 over 1116 atoms

\*Calculated using extra\_fit function within Pymol using default settings (5 cycles, cut-off = 2.0 Å) with PTPRU-D1 (reduced) as the target molecule.

**Supplementary Table 3. Primer and oligonucleotide sequences used in this study.**

Construct	Oligo (5'-3')*
PTPRU-D1 Fw	TGGC[GGTACC]CACCTGCGGTG
PTPRU-D1 Rv	TGGC[ACTAGT]CTAAGGGATGGTGGTCTCCCCAC
PTPRU-ICD Fw	TGGC[TTCGAA]CGCAAAGGGAAGCCGGTGAAC
PTPRU-ICD Rv	TGGC[GGTACC]CTATCTTGACTCCAGCCCCTCCAAGTA
PTPRU.E1053D Fw	GCCAGATCATGGCGTCCCCTAC
PTPRU.E1053D Rv	CCATGATCTGGCCACGCTGTGAAG
PTPRU.C1085S Fw	CCACAGCAGCGCGGGC
PTPRU.C1085S Rv	CTGCTGTGGATGACAATGGGCC
PTPRU.T1089A Fw	GGGCGCCGGCCGCACAGGTTGCTATAT
PTPRU.T1089A Rv	GCCGGCGCCCGCGCTGCAGTGGATGACAAT
PTPRU-D1.ΔpTyr-loop Fw	GATCGGCACCGAGTGAACTGC
PTPRU-D1.ΔpTyr-loop Rv	TTCAAAGAAGCTCTCATACTCCTGCTTGAAG
PTPRU-D1.pTyr loop oligo Fw	CTTTTTTGAAGGCTGGGACGCCACAAAGAAGAAAGACAAGGTCAAGGGCAGC CGGCAGGAGCCAATGCCTGCCTATGATCACT
PTPRU-D1.pTyr loop oligo Rv	AGTGATCATAGGCAGGCATTGGCTCCTGCCGGCTGCCCTTGACCTTGTCTTT CTTCTTTGTGGCGTCCCAGCCTTCAAAAAAG
PTPRU Exon 1 sgRNA Fw	CAGATCATAGTGCAGGGCT
PTPRU Exon 1 sgRNA Rv	AACTTGGCATTCACTCGGA
PTPRU Exon 14 sgRNA Fw	TGGGCCCTGTGCTATAGGT
PTPRU Exon 14 sgRNA Rv	AAACAGTCCCAGCAGGCATA
PTPRK-ICD Fw	TGGC[TTCGAA]AAAAAGAGCAAACCTTGCTAAAAAACGCAAAGATG
PTPRK-ICD Rv	TGGC[GGTACC]CTAAGATGATTCCAGGTACTCCAAAGCTACATCA
PTPRK.D1057E Fw	CTGGCCTGAACATGGAGTGCC
PTPRK.D1057E Rv	CATGTTTCAGGCCAGCCCGTG
PTPRK-D1.ΔpTyr-loop Fw	AATGCCTGCCTATGATCACTCCAGAGTGATTTTGAACCC
PTPRK-D1.ΔpTyr-loop Rv	CGTCCCAGCCTTCAAAAAAGCTCTCATATTCCTCTTTGAACCCATAG
PTPRK-D1.pTyr loop Fw	AGTATGAGAGCTTCTTTGAAGGACAGTCAGCATCTTGGGATGTAGC
PTPRK-D1.pTyr loop Rv	AGTTTCACTCGGTGCCGATCATATGCTATAATGTTTCCATATCGGTTTTTTGCT CTATTTTG

\*Restriction endonuclease sites are marked in parentheses

## Supplementary References

- 1 McParland, V. *et al.* The metastasis-promoting phosphatase PRL-3 shows activity toward phosphoinositides. *Biochemistry* **50**, 7579-7590 (2011).
- 2 Madeira, F. *et al.* The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res* **47**, W636-W641 (2019).